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# Effect of different storage temperature regimes on spore viability of *Paecilomyces lilacinus* (Thom.) Samson in some formulations

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**ABSTRACT:** **A laboratory experiment was carried out to identify optimum formulation medium and temperature for storage of *Paecilomyces lilacinus* based on spore viability. *Paecilomyces lilacinus* spores were produced on PDA, paddy grain, sorghum grain and tapioca broth, formulated on coconut coir pith, talc and vermiculite and stored at 0, 15 and 27 ± 1°C for 150 days, with *P. lilacinus* spores stored at -20°C on sterile silica grain as control. In general, the spore viability in grain based formulations (paddy and sorghum) declined more rapidly than on tapioca broth based formulations. The spores stored at -20°C on sterile silica grain recorded 98 per cent viability even at 150th day of storage. Spores formulated on tapioca broth with talc, vermiculite or coir pith as inert base, and on paddy grain with talc as inert base, exhibited more than 80 per cent viability at 150 days of storage at 0°C temperature which declined to ≥ 60 per cent at 27 ± 1°C after 150 days of storage.**

**KEY WORDS:** Formulations, *Paecilomyces lilacinus*, spore viability, storage temperatures

*Paecilomyces lilacinus* (Thom.) Samson is a widely worked potential antagonistic fungus that is highly effective against root-knot, reniform and citrus nematodes in different crop rhizospheres. Its commercial use is restricted to a few countries in South-East Asia and Australia. In India, *P. lilacinus* was formulated in small scale on leaf, oil cakes, grains, aqueous extracts, etc. (Mani and Anandam, 1989; Rao *et al.*, 1998). However, studies on formulating *P. lilacinus* on a specific medium that is convenient for storage, optimum storage temperature and spore viability in these formulations are lacking. For commercial production, storage and quality control aspects of any bioagent, information on optimum storage, medium for formulation and spore viability at specific storage temperatures are essential. Keeping these facts in view, a laboratory experiment was carried out to identify suitable formulation medium and optimum temperature for storage based on spore viability for nematode antagonistic fungus, *P. lilacinus*.

## MATERIALS AND METHODS

Local isolate of *Paecilomyces lilacinus* (IIHRPL1) was maintained on potato dextrose agar (PDA) at 10°C. The media tried were PDA, paddy grain, sorghum grain and tapioca broth (10 per cent). The media were autoclaved and inoculated with 1 ml aqueous spore suspension of *P. lilacinus* and left for complete sporulation at 27 ± 1°C. After

completion of sporulation, each medium was air dried at 35°C for 12-18 hours and powdered using waring blender under aseptic conditions. The inert materials selected for formulation, talc (Grade 5), coir pith and vermiculite (Grade 4) were heated in hot air oven at 70°C for three days using metal pans. The inert materials were sterilized in open pans in an oven, and not in an autoclave, in order to ensure complete dehydration of the material. Twenty-five grams of powdered PDA, paddy grain and sorghum grain media (spore laden) were thoroughly mixed with 75 g of talc powder using pestle and mortar, placed in autoclaved conical flasks in airtight polythene bags and stored at 3 temperature regimes, 0, 15 and 27± 1°C. Twenty five ml liquid (tapioca broth) culture of fully sporulated *P. lilacinus* was mixed with 75g talc powder, coconut coir pith and vermiculite in separate conical flasks and stored at the 3 temperature regimes in the same manner as given above. To all these formulations, 1 ml of 0.05N HCl and 0.5g of carboxy methylcellulose was added and mixed. Further, spores from PDA were carefully scrapped and placed in a vial containing autoclaved silica grains, freeze-dried and kept at -20°C. All the formulations developed were analyzed for recording initial viable inoculum load/ g through serial dilution using PL selective medium (Mitchell *et al.*, 1987). Similarly, 1 g of material was collected from each formulation stored at different temperatures for 30, 60, 90, 120 and 150 days and the spore viability of fungal propagules was recorded by serial dilution and plated on selective medium. *Paecilomyces lilacinus* spores stored at -20°C on silica grain were used as check for assessing the spore viability.

# RESULTS AND DISCUSSION

Spore viability was recorded highest (98 per cent) at -20°C on freeze-dried silica grain even at 150 days of storage (Table 1). Spores stored at 0°C on tapioca broth formulations (talc, coconut coir and vermiculite) and paddy grain + talc formulation showed ≥ 80 per cent viability at 150 days of storage which declined with increase in storage temperatures, i.e., about 60 per cent viability at 27 ± 1°C at 150 days on both paddy grain and tapioca broth + vermiculite formulations. Among the three tapioca broth based formulations, i.e., talc, coir pith and vermiculite, coir pith formulation behaved more stable with 74 per cent spore viability even at 27 ± 1°C after 150 days of storage.

Spore viability rapidly declined with increase in storage time at all the three temperature regimes (0, 15 and 27 ± 1°C) in PDA and sorghum grain formulations. Generally the spore viability in grain based formulations declined more rapidly than on tapioca broth based formulations. This could be due to more uniform and higher moisture levels in liquid based formulations than in grain based formulations.

It is concluded that higher spore viability was retained for 150 days at 27 ± 1°C (room temperature) when *P. lilacinus* was multiplied on tapioca broth and formulated on coconut coir pith or talc.

Table 1. Spore viability of *P. lilacinus* stored at different temperatures on different formulations

| Formulation | Storage temperature (°C) | Spore viability (%) at different days of storage* | | | | |
|---|---|---|---|---|---|---|
| | | 30 | 60 | 90 | 120 | 150 |
| PDA + Tale | 0 | 98 | 93 | 62 | 46 | 38 |
| | 15 | 94 | 80 | 51 | 35 | 30 |
| | 27±1 | 86 | 72 | 44 | 28 | 22 |
| Paddy grain + Talc | 0 | 98 | 96 | 88 | 84 | 82 |
| | 15 | 98 | 90 | 80 | 72 | 70 |
| | 27±1 | 95 | 88 | 64 | 64 | 60 |
| Sorghum grain + Talc | 0 | 98 | 98 | 90 | 88 | 82 |
| | 15 | 90 | 90 | 62 | 40 | 40 |
| | 27±1 | 82 | 68 | 36 | 20 | 20 |
| Tapioca broth + Vermiculite | 0 | 98 | 98 | 90 | 88 | 82 |
| | 15 | 96 | 94 | 86 | 80 | 74 |
| | 27±1 | 96 | 90 | 80 | 80 | 70 |
| Tapioca broth + Talc | 0 | 98 | 94 | 90 | 80 | 80 |
| | 15 | 98 | 96 | 84 | 82 | 72 |
| | 27±1 | 96 | 90 | 80 | 80 | 70 |
| Tapioca broth + Coir pith | 0 | 97 | 96 | 92 | 84 | 84 |
| | 15 | 96 | 96 | 92 | 86 | 80 |
| | 27±1 | 96 | 92 | 84 | 80 | 74 |
| Silica Grain | -20 | 99 | 99 | 98 | 98 | 98 |
| CD = (P=0.0.5) | - | 6.88 | 7.43 | 10.24 | 12.33 | 11.14 |

* Average of five replications and corrected to the nearest whole number.

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